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Biological Studies of Sewage Purification by Aeration

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BIOLOGICAL STUDIES OF SEWAGE PURIFICATION BY AERATION

BY

ROBBINS RUSSEL
B. S. Illinois College, 1914

THESIS

Submitted in Partial Fulfillment of the Requirements for the

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY ROBBINS RUSSEL

ENTITLED BIOLOGICAL STUDIES OF SEWAGE PURIFICATION BY AERATION.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Master of Science

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on
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PART I.

Since about the middle of the nineteenth century, in the more densely populated sections of the globe, the disposal of the world's waste - human dejecta, trade wastes, etc., has been an economic problem of immense proportions. England took the first comprehensive steps and has ever since taken the lead, especially in number of methods devised.

The removal of refuse by sewers,begun in the early sixties, has ever since been followed in densely populated centers and the final disposal of the vast accumulation of filth became at once a vital and very important question. Methods of disposal by burying, by sewage farms, by burning, pressing, chemical treatment, etc. have been tried but have not proved efficient enough to be generally adopted.

Of late years the tendency has been to recognize the importance of the role played by biological agencies and to utilize them for the scientific solution of the problem. To fairly comprehend the development of sewage disposal methods, a survey of the previous work, mainly of a bacterial, or rather a biological nature, must be considered.

The disposal of waste is a fundamental problem for all living organisms. As a body takes in food to build up its own structure, so it must also continually give off waste products, which if allowed to accumulate, become dangerous. While this is true of an individual, it becomes even more significant when

large numbers of organisms are together in a community. This fact, elementary and simple as it now seems was not comprehended to any degree until the middle of the last century.

Despite the nuisance which was created in all populous centers, by accumulations of filth in the closed and open cesspools, it was not till 1854 that the first British General Board of Health recommended "the passage of sewage into the nearest adjacent river or stream". In the late forties in London itself, cesspools went out of common use. About 1870, town sewers discharging from storage tanks at ebb tide, were instituted. Other countries were quick to follow England's example.

The first comprehensive project for the removal of town waste was established in the then very new city of Chicago. In 1855, E. S. Chesbrough designed a system for waste removal by water carriage. In 1860, Berlin, ever ready to adopt new methods, hastened to install a similar system. In 1884, Paris followed suit. So on, until now the method of water carriage of wastes, is the one successful method in general use.

With the establishment of this system the difficulty was shifted from the individual to the community. What to do with the accumulations of filth in the rivers was the question. As an example of the conditions England had to face, due to over pollution of her small slow-flowing streams, the following extract is taken from Budd, "Monograph on Typhoid Fever, 1873".

"Stench so foul had never before ascended to pollute the air. For many weeks the atmosphere of the Parliamentary committee rooms was only rendered barely tolerable by suspending before every window, blinds saturated with chloride of lime, and by the use of other disinfectants". Also,the Seine below Paris is spoken of as "black and stinking, being in active fermentation for a long distance, bubbles of gas as large as a meter across being formed". Thus almost at once, these communities were obliged to seek some other means of disposal than that of simply passing the untreated sewage into the rivers.

Until Pasteur, in 1860, proved that fermentation and putrefaction did not take place in the absence of living organisms, no advance toward a bacterial solution of the sewage purification problem was made. And it was not until 1868 that Sir Edward Frankland, a member of the Royal Commission, drew the conclusion, based upon excellent experimental results, "that by intermittent filtration, so-called, a non-putrescible effluent could be obtained". This method is also referred to as "land filtration".

Frankland's recommendations, as embodied in the report of 1869-70, found little sympathy in England. Towns attempting to apply his methods almost invariably secured poor results,due, as is now known, to variations from his directions. Frankland himself attributed his results to mechanical filtration and oxidation and seems not to have recognized the true agencies.

Foremost among the early investigators to comprehend the real cause of this purification was Dupre. His work also was conducted in England. In 1884, in a report to the local government board of England, he stated that "the consumption of dissolved oxygen in natural water is due to the presence of growing organisms". And in 1887, "Whatever scheme may be adopted, saving only destruction of the sewage material by fire, the agents bringing about the ultimate destruction are living organisms, therefore the treatment followed should be such as to avoid killing or hampering these forms."

During this same period, Emich of Germany was studying the changes brought about in sewage by aeration and by treatment with antiseptics. He concluded that the direct oxidation of organic matter by the oxygen of the atmosphere, if it occurred at all, was so slight as to be negligible. He also found that oxidation was due to minute organisms.

One of the first practical applications making use of these biological agencies was in France and not in either of the countries in which the experimental investigations were conducted. About 1882, after some twenty years experimenting, the Mouras Automatic Scavenger was brought out in France. This remarkable device consisted of nothing more or less than a closed vault, in which anaerobic decomposition occurred. It was soon found that it did not give a stable effluent, so it disappeared from public view.

At almost exactly the same time, patents were granted to E. S. Philbrick of Boston, for a series of closed tanks, similar to the one described above, followed by filters on which aeration occurred. Experimentally these were a success, but cost and the failure to comprehend the true agents of purification, along with other less direct causes, brought about the commercial failure of the enterprise.

From 1875 to 1882 England experimented almost exclusively with the sewage farm system of waste disposal, i.e., using the sewage, more or less treated, as a fertilizer. Because of its inefficiency the cost, and the nuisance so often created, this system has been in large measure abandoned. The Royal Commission of 1882 introduced chemical precipitation of the solids in sewage, along with attempted sterilization of the effluents. For the effluents so produced, suffice it to quote the words of Mr. Dibdin, one of the most noted of English sanitary chemists. "As the very essence of sewage purification is the ultimate destruction or resolution into stable combinations of the unstable, undesirable parts of waste, it is evident that an antiseptic process gives the very reverse of the desired object. Furthermore, alkaline effluents, such as are produced by chemical treatment, are very liable to putrefy, instead of becoming purified by oxidation." This method of treatment gives also, immense quantities of more or less unstable sludge, i.e., (the precipitated solids), to be disposed of,

thus tending to make the method both costly and unsatisfactory.

Frowned on by the country in which it was originally devised, Frankland's intermittent filtration was almost altogether lost sight of. It was in the United States that work along this line was first resumed.

With Frankland's data as a foundation, experiments of a really scientific nature were undertaken under the direction of the Massachusetts State Board of Health at the Lawrence Experiment Station. The object of these experiments was at first, the removal or destruction of bacteria by straining and by chemicals. Later, studies of intermittent filtration and purification by aerobic organisms were undertaken. The conclusions drawn were, "that to completely destroy and stabilize organic matter it was only necessary to provide optimum ecological conditions for aerobic bacterial life".

Worcester, Massachusetts, made use of these conclusions and constructed sixteen one-acre filter beds, using coarse sand as the medium. When not overworked these filters gave excellent effluents. The capacity of these beds is, however, limited. Furthermore all very concentrated waste must first be treated to remove suspended matter before it is run onto them. The preliminary treatment gives quantities of unstable sludge to be disposed of. The work of the Massachusetts State Board of Health, epoch making though it was, still left the following questions

undecided. First, how to bacterially treat the sewage of large cities where land and sand are not plentiful? Second, how to dispose of the suspended matter? and, third, how to treat sewage containing large quantities of trade wastes?

The Main Drainage Committee of England, in 1891, authorized a series of experiments, similar to those in Massachusetts, but under the name "biological method of treatment". These answered in part the first question left unsettled in the earlier work. By substituting coke breeze in place of coarse sand as the bed medium, enlarged capacity was obtained. Still sedimentation and even screening had to be resorted to, before filtering, leaving the sludge disposal problem still untouched. Furthermore, as shown by both Rideal and Frankland, the efficiency of these beds varies greatly.

Despite the many obvious defects of this system, many English towns adopted it either in part or entirely, but by 1896, chemical treatment of sewage was generally abandoned throughout England. In practice with the "biological method", it was found almost imperative not only to screen but also to treat anaerobically in "bacteria tanks", so-called, before running the waste on the filters. In spite of this, cost of treatment was reduced from $75 per million gallons, to about $18.

Cost, variable and often unsatisfactory results, and the nuisance so often resulting from this method of treatment, together with the dawning realization of the tremendous economic

waste involved, in cost of sludge disposal and in valuable fertilizer lost, induced further investigations. These followed the line of increasing the supply of air in the filters, i.e., creation of more optimum conditions for aerobic action.

To trace this from the beginning, it is necessary to look again to the States. In 1894, Waring at Newport, R. I., was treating sewage by forced aeration, the only preliminary treatment being that which might occur in the pipes. His system was to run the untreated sewage on to several beds of broken stone. The action here was considered to be a "mere mechanical sedimentation". When the beds became clogged the sludge was drawn off and aerated. Little is now known of the success or failure of this system.

In Cleveland, also in 1898, we find a reported reduction in ammonia of 98.8% and of bacteria of 99%, by means of double filtration through slag and coke, with aeration under light pressure.

As shown by the Massachusetts Experiment Station, the temperature of inception of active nitrification, was about 39° F. Therefore, the maintenance of bacterial-filter efficiency during winter became a rather serious problem. Many schemes were proposed to overcome this. At Leeds, England, one of the most practical, the Whittaker-Bryant thermal-aerobic filter, was installed. In this, the effluent from a septic tank was distributed over the filter bed through automatic revolving

sprinklers, a small jet of steam being blown into the sewage as it left the nozzle. The effluent from these beds, however, was stable only a few days.

Besides the intermittent filters of the types described above, contact and sprinkling filters have been evolved. In the former the chief noteworthy difference is the use of a much coarser medium with consequent increased rate of flow and lessened liability of clogging. But a single contact filter does not give an altogether satisfactory effluent. In this country, it has been shown* that with preliminary septic treatment, contact filters are able to produce non-putrescible effluents at high rates, while sprinkling filters give corresponding results at even higher rates of flow. In addition to this, the cost of operation of the sprinkling type has been found to be much lower than that of the contact beds, even when settling basins are used.

But still a truly successful method of sewage purification was far from being attained. The International Congress of Hygiene and Demography decided that the scientific solution of the sewage problem will not be attained until the following results are guaranteed for any given case:* (1) an effluent which will not deteriorate the stream into which it flows. (2) no nuisance in

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*Calvert - "Sewage Purification" from German of Dr. Dunbar.

*Intern. Cong. Hyg. and Demog. Sec. VI, 375. 1912.

the course of the sludge disposal. (3) no nuisance from flies or from smell in connection with the filter beds. (4) an expenditure strictly proportionate to the sanitary and aesthetic results achieved.

To cut down the cost and dispose of the sludge without nuisance were the two main factors urging further work. Already reasonably reliable effluents had been obtained and the fly nuisance in large measure abated. Still even in the best plants, sedimentation basins, screens, septic tanks and filters exist, all of which increase the cost of construction and maintenance. Also in most cases there is a heavy, voluminous, more or less putrescible and valueless sludge to be disposed of, which if it could be utilized would mean dollars saved.

In 1913, Clark and S. DeM. Gage published the report of their work at the Lawrence Experiment Station showing that untreated sewage, when aerated in a bottle for twenty-four hours, was nitrified to a considerable degree, in the presence of green algal growths. These same investigators found subsequently that aeration for a much shorter period in a tank containing slabs of slate about one inch apart and covered with a brown growth was sufficient to coagulate the sewage colloids, producing a non-nitrified effluent, well clarified and capable of filtration at several times the normal rate.

Fowler and Mumford in their work with M7.* a peculiar

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*J. of Royal San. Inst., 34, 497. 1913-14.

organism precipitating iron as $Fe(OH)_3$ from iron salt solutions, obtained after six hours aeration an effluent quite clear and non-putrefactive on incubation. This was nitrified readily by filtration at a high rate.

In all these experiments and the later ones on an enlarged scale, the old idea of surface contact, exemplified by the plates in the aeration tank of Clark and Gage, is retained. Also, in order to produce a nitrified and stable effluent further filtration is necessary. The processes involve several kinds of tanks, etc., and there is the serious question of the disposal of large quantities of unstable sludge.

In November 1912, Dr. Fowler returning from a visit to the Lawrence Station, with the above mentioned experiments of Clark and Gage in mind, started two men, Ardern and Lockett* experimenting with sewage disposal by aeration. Inasmuch as the work is new and unique, a brief summary of their conclusions will be given.

Preliminary experiments in 80 oz. aeration bottles, were run to obtain nitrification of Manchester raw sewage. When completely nitrified, for the first sample at the end of five weeks, the effluent was siphoned off and fresh sewage placed in contact with the original deposited matter. Less time was required for nitrification at each treatment until complete

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*J. Soc. Chem. Ind., 33, 523-39; 1122-24. 1914.

nitrification of raw sewage was obtained in six hours. As soon as a sufficient quantity of the "activated sludge" so-called, was obtained, experiments on various types of sewages were undertaken. It was found among other things that the rate of nitrification depended in some measure on the concentration of the sewage.

In every case, a rapid initial effect on the oxidizable matters (colloid and crystalloid) was noted. This occupied about three hours and gave a well clarified effluent. The latter stages of the aeration were largely confined to the nitrification of the ammonium compounds.

The sludge accumulated in this process is quite inoffensive and though of a very low specific gravity, settles from the effluent at a very rapid rate.

It is to be noted also that while immense numbers of bacteria, around thirty million per cc. (on gelatine at 20°C.) and also a varied fauna of protozoa exist in the sludge, no algal growths were found at any time. The final conclusions from these experiments are:

"1. The resultant solid matter obtained by prolonged aeration of sewage has the property of enormously increasing the purification effected by simple aeration of sewage, i.e., intensifies the oxidation process.

2. The intimate manner in which the activated sludge is brought in contact with, and its proportion to the amount of sewage bears a direct relation to its accelerating effect.

3. In order to maintain the sludge at its highest efficiency it is necessary that there should not be at any time an accumulation of unoxidized sewage solids. Note - the activity of sludge may be maintained for a time, at least, by suitable aeration of the sludge alone.

4. Temperature exerts a considerable influence on the oxidation. Between 10 and 24 degrees centigrade there is no material difference in the efficiency of the sludge, but higher temperatures interfere. It was noted though, that nitrification itself was stimulated by the higher temperatures.

5. The period of treatment depends upon the "strength" of the sewage, degree of purification desired, etc.

6. Activated sludge differs markedly in character from ordinary sewage sludge. It is well oxidized, consequently innocuous, can be readily drained on straining filters and possesses a high nitrogen content."

These conclusions were based entirely on laboratory experiments*, but in those on a larger scale fairly similar results have been obtained.

As a result of studies on the possibility of a continuous flow treatment, instead of the "fill and draw" method used, it became clearly apparent that satisfactory progress with this method of treatment, was dependant upon the design of a tank,

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*J. Soc. Chem. Ind. 33, 523-39; 1122-24. 1914.

which without increasing the mean rate of flow would allow a certain minimum period of aeration sufficient to produce a non-putrescible, clarified effluent. Investigation has shown that this minimum period ranges from four to six hours. Continuous flow plans have been temporarily at least, given up.

The above experiments, which have been repeated and studied at Illinois*, have served to confirm the above results.

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*J. Ind. and Eng. Chem. 7, 318. 1915.

But since a thorough knowledge of the character and mechanism of the changes taking place is essential for the proper development, operation and control of the process, studies both of the mechanical devices and the physical, chemical and biological processes entering into the action of the process, are now going on. The work, described below, was undertaken primarily to find out whether a consistent reduction in the number of bacteria in the effluent is obtained, and what bacteria or other organisms cause the purification.

Unless special mention is made, all media is prepared in accordance with Standard Methods as published by the American Public Health Association, 1912.

Examination of Effluent. Counts on the number of bacteria present are made by the dilution method. Dilutions are made on the 10 to 100 basis, sterile distilled water being the medium. Each flask is thoroughly shaken (30 times) before taking

a sample or diluting further. In running dilutions the minimum of time possible was used.

The original sample for examination is drawn directly from the acting tank, a wide mouthed two-liter bottle being filled with the mixture of sewage and sludge. This is then allowed to settle for one hour and a ten cc. sample of the supernatant liquid withdrawn for analysis, care being taken to insert the tip of the pipette the same depth each time. This method of collection is followed since in the actual process, settling precedes the drawing off of the effluent. The mixture in the aerating tank is so intimate that the variation in the comparative amounts of sludge present in the two-liter sample from day to day with a possible variance in the number of bacteria taken out by the settling action are negligible. Preliminary examinations on the tank showed, that while running, almost identical conditions exist throughout the whole mass of sewage and sludge.

Whether a consistent reduction in the number of bacteria in the effluent is obtained after treatment was investigated by means of a series of counts running from January to May of the present year. See Tables I and II. An average reduction in the agar count from 750,000 per cc. in the raw sewage, to 40,000 per cc. in the effluent at the end of a four hour aeration period is shown. On gelatine a reduction from 4,000,000 to 200,000 per cc. is noted. The average counts for the number of bacteria in the raw sewage are higher and for the number present

TABLE I.

REDUCTION IN BACTERIA.

Growth on Agar -- 37°.

Date	Sludge A in bottle	Sludge B in tank	Raw Sewage	Aeration 1 hr.	Aeration 2 hrs.	Aeration 3 hrs.	Aeration 4 hrs.
Jan. 21	Rain		248,000	58,500	---	---	---
Mar. 10	----	---	800,000	---	---	---	194,000
17	565,000	3,495,000	660,000	700,000	145,000	81,500	Note: counts on eff. from tank
22	1,115,000	4,200,000	Note: sludge in bottle regaining efficiency now.				
24	----	3,500,000	765,000	---	---	---	---
Apr. 10	----	---	750,000	750,000	200,000	109,000	65,000
12	----	2,800,000	790,000	755,000	210,000	105,000	52,000
14	----	----	800,000	720,000	175,000	102,000	58,000
17	----	----	1,000,000	500,000	210,000	55,000	24,000
19	----	2,450,000	840,000	640,000	224,000	97,000	71,000
20	----	----	620,000	590,000	240,000	105,000	68,000
28	----	1,470,000	760,000	340,000	170,000	47,000	13,000
30	----	400,000	740,000	385,000	195,000	69,000	Note: poor eff. from tank.
May 3	----	2,100,000	610,000	625,000	226,000	116,000	33,000
4	----	2,000,000	716,000	614,000	190,000	110,000	79,000
6	----	2,100,000	720,000	600,000	220,000	84,000	26,000
10	----	----	790,000	545,000	188,000	92,000	19,000
12	----	2,200,000	660,000	590,000	210,000	74,000	22,000

TABLE II.

REDUCTION IN BACTERIA.
Growth on Gelatine - 20°.

	Sludge A in bottle	Sludge B in tank	Raw Sewage	Aeration 1 hr.	Aeration 2 hrs.	Aeration 3 hrs.	Aeration 4 hrs.
Mch. 10	----	----	7,000,000	----	----	----	----
17	8,000,000	10,300,000	6,000,000	2,220,000	850,000	252,000	----
22	11,140,000	19,000,000	----	----	----	----	----
24	----	16,000,000	6,800,000	----	----	----	----
Apr. 10	----	----	7,000,000	3,650,000	1,040,000	314,000	255,000
12	----	15,600,000	7,000,000	3,200,000	1,600,000	320,000	240,000
14	----	----	5,500,000	1,850,000	1,000,000	310,000	220,000
17	----	----	2,500,000	215,000	360,000	165,000	18,000
19	----	13,000,000	6,550,000	2,100,000	1,270,000	243,000	201,000
20	----	----	4,200,000	2,800,000	1,110,000	260,000	230,000
28	----	6,000,000	4,000,000	1,270,000	820,000	196,000	121,000
30	----	700,000	3,800,000	1,740,000	1,100,000	430,000	----
May 3	----	7,480,000	4,800,000	2,960,000	1,370,000	387,000	217,000
4	----	8,600,000	3,960,000	2,180,000	1,100,000	265,000	200,000
6	----	7,600,000	4,100,000	1,860,000	1,140,000	269,000	233,000
10	----	----	3,740,000	1,960,000	1,080,000	340,000	254,000
12	----	8,250,000	4,270,000	2,140,000	1,260,000	370,000	260,000

in the effluent after treatment, much lower than those given. Eleven hours aeration shows on agar, 27,000 per cc. and on gelatine, 102,000. Inasmuch as these counts cover a period of four months with the consequent variations due to weather, dilution, etc., that this process does give a consistent large reduction in the number of bacteria present in sewage is proven.

The reduction seems to be caused, in large measure, by the mechanical "sweeping out" of the effluent, by the rapidly settling sludge which resembles very much in its action a very flocculent precipitate like $Al(OH)_3$. The destruction of bacteria by the other inhabitants of the tank is not to be overlooked in accounting for this reduction, inasmuch as bacteria form one of the staple articles of their diet. Other causes, now unrecognized, may also play a part.

An idea of the proportion of the total reduction, that can be attributed to the settling sludge, may be gained from this table. These counts were made on April 10, 17, and 20 of this year.

Media	Agar	Agar	Agar	Gelatine	Gelatine	Gelatine
Raw Sewage	750,000	1,000,000	620,000	7,000,000	2,500,000	4,200,000
Effluent after settling	210,000	360,000	300,000	1,140,000	1,800,000	2,400,000

The samples for this examination were taken in exactly the same manner as described before. They were drawn five

minutes after aeration of the sewage and sludge in the tank was begun. As a comparison with the figures on the total reduction in the number of bacteria, (See Tables I and II), there are undoubtedly other agents entering into this reduction since the settling of the sludge accounts for less than half of the total.

To ascertain the rate of reduction of B. coli (by this term being meant, acid and gas formers growing at 37°) seven series over a period of almost a month/ were run. (See Tables III and IV).

In Table III, reduction in the number of acid gas formers shown by growth in standard lactose broth, +1 cc. of 1% azolitmin solution, in Smith fermentation tubes, is shown. Table IV. gives series run parallel to those in Table III as a check, standard lactose agar with 1 cc. of the azolitmin solution added, being used. A reduction in the number of these acid-gas formers, from 90 to 99% was invariably noted. Comparison with the counts given in Table I shows further that from 80 to 95% of the bacteria (agar 37°) found in Champaign sewage, are acid gas formers.

In this long series of counts made under widely varying conditions of temperature, density of sewage, etc., a consistent reduction in the total number of bacteria present as shown by tests on standard media and an even greater percentage reduction in "acid gas formers" as indicated by cultures in litmus lactose agar and broth, was invariably obtained. The results justify the conclusion that stable effluents, produced by this process, show a uniform and consistent reduction in all kinds of bacteria. No

TABLE III.

REDUCTION OF ACID AND GAS FORMERS.
Growth in Litmus Lactose Broth - 48 hrs, 37°.

Apr.	Raw Sewage	Sludge and Sewage	Effluent aerate 1 hr.	Effluent aerate 2 hrs.	Effluent aerate 3 hrs.	Effluent aerate 4 hrs.
10	Between 1,000,000 100,000	Between 10,000,000 1,000,000	Between 1,000,000 100,000	Between 1,000,000 100,000	Between 100,000 10,000	Between 10,000 1,000
14	Between 1,000,000 100,000	----	Between 1,000,000 100,000	Between 1,000,000 100,000	Between 100,000 10,000	Between 10,000 1,000
17	Between 1,000,000 100,000	Between 10,000,000 1,000,000	Between 1,000,000 100,000	Between 1,000,000 100,000	Between 100,000 10,000	Between 10,000 1,000
19	Between 1,000,000 100,000	Between 10,000,000 1,000,000	Between 1,000,000 100,000	Between 1,000,000 100,000	Between 100,000 10,000	Between 10,000 1,000
May 1	Between 1,000,000 100,000	----	Between 1,000,000 100,000	Between 1,000,000 100,000	Between 100,000 10,000	Between 10,000 1,000
4	Between 1,000,000 100,000	----	Between 1,000,000 100,000	Between 1,000,000 100,000	Between 100,000 10,000	Between 10,000 1,000
6	Between 1,000,000 100,000	----	Between 1,000,000 100,000	Between 1,000,000 100,000	Between 100,000 10,000	Between 10,000 1,000

TABLE IV.

REDUCTION IN ACID AND GAS FORMERS.

Growth on Litmus Lactose Agar - 24 hours, 37°.

Date	Raw Sewage	Sludge and Sewage	Effluent aerate 1 hr.	Effluent aerate 2 hrs.	Effluent aerate 3 hrs.	Effluent aerate 4 hrs.
Apr. 10	600,000	1,400,000	500,000	160,000	20,000	6,000
14	550,000	--	500,000	140,000	30,000	10,000
17	700,000	1,400,000	400,000	180,000	10,000	8,000
19	720,000	900,000	520,000	160,000	26,000	7,000
May 3	540,000	---	530,000	140,000	18,000	5,000
4	620,000	---	515,000	185,000	29,000	6,500
6	680,000	---	580,000	196,000	37,000	6,200

claims are made for a "bacteria free" effluent. Merely that in this process, unlike other bacterial purification methods in vogue, a real reduction in bacteria is obtained. No work has been undertaken to determine the effect of this process on disease producing parasites. Since these are present, if at all, in small numbers, it seems reasonable to hold that the same "rate of reduction" applies also to them, with a consequent proportionate lessening of the danger of infection.

PART II.

The second part of this investigation was undertaken with the object of finding out, if possible, - first, the various agents playing a part in the purification process, and second, the various roles with their relative importance in the whole process.

To identify these agents, an extensive examination of the tank's population (flora and fauna) was undertaken. Note: In the list of forms considered, are those constantly appearing throughout the examination. It was hoped in this way to secure only those really playing a part in the process.

That the purification is entirely due* to life in the media was shown very easily. Fifty cc. of "activated sludge" were taken from the tank, to this was added an equal volume of N/250 ferrous sulfate. After twenty-four hours, complete absence of life, as observed under the microscope, resulted. Examination was made on three samples. Raw sewage in proportion of 4 to 1, was then added and the mixture subjected to twenty-four hours aeration. The mixture of sterilized sludge and sewage had the same concentration as that existing in the experimental tank. In order that the sludge's composition might be as slightly altered as possible, it was sterilized in flowing steam for periods of thirty minutes each on three successive days. After twenty-four hours aeration of this mixture, examination showed the ammonia to be lessened (due

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*J. Soc. Chem. Ind., 33, 523-39; 1122-24. 1914.

probably to the passage of air) but no nitrification.

The above experiment having definitely shown that the population of the tank was responsible for the changes taking place, a detailed study of this population was undertaken. As too little was known concerning the functions performed by the various inhabitants to indicate the study of one particular form, they were taken up as they came. The first considered was the macroscopic non-bacterial flora. The work at best was only roughly qualitative because of the difficulty of securing them in pure culture. Also, while this work was still under way other experiments, described later on, showed clearly that the function of the macroscopic, non-bacterial forms in the nitrification process was negligible.

Of the non-bacterial flora, the most noticeable is a relatively large worm,* 1 - 5 mm. in length, found everywhere throughout the medium. This form is an annelid of the class - Oligochaetae, known as Aelosoma hemprichii. Excellent growth of the form was obtained, employing a 1/10% wheat extract, aerated as the medium.

The movement of the worm, always anterior, is of two kinds, i.e., when in a current it retains its position by means of two large caudal papillae, moving with a distinct looping motion, but when in quiet surroundings, as in a counting cell, its movements are of a gliding nature, cilia being the organs of propulsion. Its food consists of "bacteria, diatoms, unicellular-algae

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*J. Ind. and Eng. Chem. 7, 320. 1915.

and the mesophyl tissue of decaying leaves".* Concentrations of organic and mineral acids, as low as N/2000 prove fatal almost instantly as does also N/500 alkali. In fact, the worm's resistence to any foreign toxic substance is extremely slight. That this fact accounts in large measure for their disappearance at times, due to the coming in with the sewage of some toxin, cannot be doubted.

An attempt to secure a pure or almost pure culture of worms was made in the following manner: From the synthetic wheat broth, about two hundred worms were withdrawn in a pipette. These were then filtered. On this filter, the worms were rapidly washed ten times with sterile distilled water, it being hoped that extraneous bacteria would be largely removed. The worms were then quickly inoculated into ammonia broth, enough sterile sludge being added to bring the specific gravity of the mixture up to that of the tank sludge. This mixture was then aerated in a flask of the following design:

A wide mouthed two-liter flask is fitted with a three hole rubber stopper, each of the two outer holes carrying a piece of glass tubing bent at right angles.

2, reaches to the bottom, a long piece of sterile rubber tubing

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*For further discussion see Jour. of Exp. Zoology, 8, 43. 1910.

perforated in many places, with the outer end solid, being attached at its lower extremity. 3 extends just through the stopper. A three-way stop cock is run down through the third hole, with one limb, 4, extending to the bottom of the flask; another, 5, is bent at right angles, while on the third, 6, a bulb of about 10 cc. capacity, is blown. 2, 3, and 6, are plugged with cotton, and the whole sterilized. By means of the stopcock, 6, can be put into communication with 4, and a sample drawn into the bulb; then by turning the stopcock this sample can be run off through 5 for analysis. This apparatus, adapted from Beesley* will be referred to throughout this paper as apparatus A. In every case extremely satisfactory results were obtained by its use.

When it is desired to aerate the solution, by simply attaching to 2 a series of wash bottles containing in order from the flask, cotton wool, sterile water and c.p. sulfuric acid, this can be aseptically and easily accomplished.

In this apparatus, the worm culture above mentioned, was aerated for one week, daily examinations both microscopic and chemical being made. These are given in the following table. (+ indicates worms are living)

Day	1	2	3	4	5	6	7
NO_2 p.p.m.	0	0	.1	.1	.1	.2	.4
Worms	+	+-	+	+	±	---	---

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*Jour. Chem. Soc., 105-106 - 1014. 1914.

The worms had disappeared on the sixth day.

About the only conclusion which can be drawn from the above almost self explanatory data, is that the worms themselves have no power of oxidizing ammonium salts. On the last day a sample of the nitrifying broth was plated out on synthetic media. Suffice it here to say that bacterial growth, identical with the nitrifiers, later discussed, was obtained. These undoubtedly were introduced with the worms. The slowness of nitrification may have been due to one or both of two causes; paucity of the nitrifiers inoculated with the worms, and the possible restraint of their growth, by attacks of the worms. Because of the failure of this attempt to secure a pure culture of the worms, the problem of the exact role which these animals play in the purification process was left in order to study the part taken by the bacteria.

It may be of interest, however, to give the results of some work just completed in England by Jas. Crabtree*, under the direction of Dr. Fowler, on the function of the non-bacterial population of contact beds.

"1. In normal beds, protozoa, etc. satisfy their hunger on the fresh bacteria brought into the bed by the sewage, thus allowing the normal population to carry on its work of purification undisturbed.

2. The change in the bacterial population can never

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*J. Royal Sanitary Institute, 34, 493. 1914.

become marked, however, because the fresh sewage, rich in all manner of forms, keeps the population nearly normal.

3. It is extremely improbable that the animal population influences the soluble constituents in the sewage directly."

He finds that in contact beds and even more so in sprinkling filters, the animal population is advantageous in,

"1. Maintaining the capacity of the bed by keeping it 'open'.

2. In keeping down extraneous bacteria.

But this population plays no direct part in the oxidation processes going on in the beds."

After an examination over a period of forty days, the object of which was to determine among other things, the typical fauna inhabiting this process, if any such could be said to exist, suffice it for the purposes of this paper to say that Vorticella and Rotifers constitute the major portion of the non-bacterial population, aside from worms. A superficial examination classifies the former under class Infusoria; Order, Ciliata; the Rotifers under Class Rotifera, Order, Bdelloida (with tail, and Trochosphaerida (without tail). Of algae there were none. In addition to the above given types many others appeared sporadically, of which no record was kept.

No experiments were attempted with these forms, but an experiment described later in the bacterial discussion, shows their

role in the nitrification process, if they have any, to be a negligible one. As a result of this study it was shown that algae took no part in this process, contrary to the tentative suggestion of Clark and Gage at the Lawrence Experiment Station,* that the stabilization obtained in their experimental tank containing the slate slabs was due to algal growths.

Having thus disposed of the macroscopic, non-bacterial fauna, only the bacteria can be considered as important agents in this process, for no evidence was found to indicate that the numerous protozoan forms, Paramoecium, etc., which at times appear, have aught to do with the process, aside from the destruction of bacterial life, etc. Excellent results were obtained, the experiments being given later, in the absence of all protozoan life; so the function of these also, is such exists in the stabilization processes, is almost negligible.

The question of the exact function of the various members and groups, making up the bacterial population, in sewage purification processes, is one which has been studied for over twenty-five years. It was only recently, however, that clear connected ideas began to be advanced.

Pasteur first suggested that oxidation of ammonia to nitric acid was really due to micro-organisms, and two French chemists, Schlosing and Müntz first actually proved this to be

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*Bull. Lawrence Expt. Station. Mass. State Board of Health. 1912.

the case. It was not until 1889, however, that any successful work on the specific organism causing nitrification was reported. Warington, despite his excellent and exhaustive study of the conditions under which nitrification occurs, was never successful in isolating the specific organism causing this action.

Almost simultaneously, Winogradski in Russia* and Percy Frankland in England,** succeeded in isolating the specific organism making nitrites from ammonia, employing gelatinous silica as the medium for cultivation. The nitric organism was more difficult to isolate, but in 1891, Winogradski succeeded.

The direct application of this discovery, to the explanation of contact and other bacterial-bed's actions, has been worked out of late years by Boullanger and Massol of the Pasteur Institute at Lille,*** by Schulze-Schulzenstein in Germany, and by Dr. Harriette Chick of the Lister Institute,° England. All these investigators agree that the nitrifying organisms found in ordinary

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*Ann. de l'Inst. Pasteur, 4, 213,257,760. 1890.
Ann. de l'Inst. Pasteur, 5, 92,577. 1891.

**Phil. Trans. Roy. Soc. B. 1107. 1890.

***Calmette et Confreres, I, 89. 1898.

°Bact. and Enzyme Chem. Fowler. 266. 1911.

sewage filters are the same as those occurring in the soil. The question of how these organisms, which Winogradski had so conclusively shown could not maintain their activity in the presence of organic matter, nitrified sewage products, with all the organic matter necessarily there, was first explained by Boullanger and Massol. They conclude that while the above conditions may impede the original growth and development of the nitrifying organism, yet when it is once established, its activity is unaffected by considerable concentrations of organic matter. This is experimentally borne out by the well recognized necessity of "ripening" a new filter, before submitting it to its full capacity, otherwise extremely poor results are obtained.

Dr. Chick further determined that in sewage filters, as in the experiments with solutions, nitrification takes place in two distinct stages, first nitrites then nitrates being formed.

And finally, the work of Dr. Fowler and Percy Gaunt* on the effect of surface on the rate of nitrification, showing that not only the amount of surface per unit volume of sewage, but also the character of that surface has a marked effect. Much better results are obtained with porous, irregular material, such as broken clinker, than with a hard medium such as quartz.

In the particular method of treatment under discussion here, it is desired to know why it is that excellent sewage

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*Bact. and Enzyme Chem. Fowler, 227. 1911.

purification is obtained by aeration in the presence of sludge without preliminary treatment of any sort.

The consideration of this question involves two principle factors: first, what takes the place of the preliminary treatment hitherto found necessary in preparing sewage products for nitrification, and second, how is it that in a tank with no solid medium for the growth of the nitrifiers, complete stabilization and at least partial nitrification are secured in four hours?

Before a definite study of these conditions and their causes can be undertaken, the specific organisms taking part in the reaction must be known. The identification of these organisms was therefore undertaken. It was hoped to determine if anything similar or comparable to a "typical flora" with certain definite functions might exist in the sludge, also to find out if the regular nitrifiers mentioned above are present.

Media used for the study was made in accordance with the following directions: Silica Jelly -* First, to learn percent silicic anhydride in sodium silicate; decompose the silicate with conc. hydrochloric acid, evaporate to dryness and wash till the water shows no chloride. Heat about 25 grams to redness and determine the silicic anhydride.

Second, dilute the silicate to be used till the solution contains from four to five percent of silicic anhydride.

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*Centralblatt fur Bakt. - 2 Abt. 21, 84.

Third, prepare standard hydrochloric acid of such strength that one cc. exactly neutralizes one cc. of the silica solution, methyl orange being the indicator.

Fourth, to 104 cc. of the acid, add slowly and with constant stirring, 100 cc. of the silica solution.

Fifth, tube and sterilize at 120° for 15 minutes. The solution should come out clear. If turbidity appears, it shows a deficiency of acid. In order to cause the solidification of this medium, add to each tube 1 cc. of such sterile solution of salts as is employed, in every case with enough sodium carbonate to little more than neutralize the acid present. If making plate cultures inoculate the base before adding the salts. Magnesium carbonate gives results equally as good as those with sodium carbonate, but a pure clear solid media is more difficult to obtain.

Synthetic Broth - for the nitrite bacteria.

$(NH_4)_2SO_4$ or $(NH_4)_2CO_3$	1 gr.
KH_2PO_4	1.0 gr.
$MgSO_4$	.5 gr.
$Fe_2(SO_4)_3$	.4 gr.
NaCl	2.0 grs.
H_2O distilled	1000. cc.

Dissolve the KH_2PO_4 separately. Add 10 grs. of reprecipitated $CaCO_3$, sterilized separately. Sterilize in flowing steam, 30 min., 2 days.

Synthetic Broth - for the nitrate bacteria.

$NaNO_2$	.5 gr.
KH_2PO_4	.5 gr.
NaCl	.5 gr.
$Fe_2(SO_4)_3$	.4 gr.
$MgSO_4$	.5 gr.
H_2O distilled	1000. cc.

Same additions and directions as above.

Synthetic Agar: For the forms desired, choose the suitable one of the two above given broths. Before adding the sterile $CaCO_3$, add 1.5% of pure agar. (Prepared by soaking in fresh water till the butyric acid fermentation appeared.) Sterilize and add the $CaCO_3$.

Gypsum block: To 200 grs. of crude plaster of paris ($CaSO_4$) **and** 2 grs. of $MgCO_3$, add water slowly till a mass with the consistency of wet plaster results. With this make a layer about 1/4" thick on a glass plate, and before hardening, mark off into squares about two inches on a side. When hard, pry loose and put into petri dishes. Sterilize with steam at ten pounds pressure for ten minutes. When cool, carefully add sterile ammonia or nitrite broth (see above) till the blocks are half covered. Inoculate with four or five loopfuls of the material under investigation.

Experimental. - In one aerating apparatus (see apparatus A), four hundred cc. of sterile "ammonia broth" to which was added 10 cc. of "activated sludge", was placed and aerated till active nitrification was obtained. In another flask, four hundred cc. of "nitrite broth", similarly inoculated, was aerated as above. When strong reactions were secured in both flasks, ten cc. from each was inoculated into fresh flasks containing similar broth, this being repeated till, in accordance with the well known "accumulation method" of Beijerinck, almost pure cultures of the nitrifiers were obtained. These were then inoculated on silica jelly plates and incubated at 37°C. Growth occurred in about twelve days. From these colonies, fresh silica jelly and synthetic agar plates were poured for both the nitrite and nitrate bacteria. The former was also inoculated on gypsum blocks. Samples of each of these were incubated both at 37°C and 20°C.

In nine days, slight growth of the nitrite organism on the synthetic agar at 37° was found. Two days later, the nitrate form appeared. Growth of both was secured on silica jelly, but was much slower and more scanty. Of all the gypsum blocks incubated, only one gave growth. The failure of these last as a medium for growth, was due, as was later learned, to a too great concentration of the magnesium in the media.

The colonies, as they appear on silica jelly, where they are most easily seen, are small slightly yellowish irregular

growths. There seems to be no characteristic form to them.

Microscopic examination of these forms showed, for the nitrite bacteria, a fairly large, short, rod-shaped organism. By comparison with forms from the soil this was found to be without doubt the typical "nitrosomonas, . the nitrite organism of soil. The nitrate organism is found to be a very small bacterium, only slightly longer than broad, conforming in every respect to the common "nitrobacter" of soil bacteriology.

To properly identify organisms as the causative forms in a process, according to the postulates of Koch,* the form should first be isolated, then reinoculated into fresh material, the same effect secured as was originally observed and the organism again isolated from this new culture.

Fresh sewage coming directly from the Champaign sewers, was sterilized in flowing steam, in 200 cc. portions for thirty minute periods on two successive days. Examination showed a sterile medium. To one aerating flask containing this sterilized sewage (see apparatus A) a strong innoculum of "nitrosomonas" from the synthetic agar culture, was added. Into another flask, nitrobacter was inoculated, while in a third, a mixture of both these forms was placed. A fourth apparatus was run in blank as a check. Because of the size of aerating flasks, it was necessary to carry on all experiments at room temperature. Air, previously

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*Bull. 249, N. Y. Ex. Station, by Harding.

washed as described above, was passed continuously through the apparatus. At this same time, a fifth apparatus was set up, exactly similar to the above, but inoculated with 2 cc. of the "activated sludge" instead of the pure nitrifiers.

These were all tested at the end of twenty-four hours aeration, with the following results: The first flask containing only nitrosomonas showed between .1 and .2 p.p.m. of nitrite more than was shown in the fourth flask, the blank. The colorimetric test with α napthalene hydrochloride and sulfanilic acid was employed. The second flask showed no nitrates and the same nitrite content as the sewage in the blank. The third flask, containing a mixture of nitrosomonas and nitrobacter gave traces only of nitrites and nitrates. The fifth flask, inoculated with activated sludge gave 12 p.p.m. of nitrates and no nitrites or ammonia. From each of the above flasks, the respective organisms were again obtained. This brought up the question, why was the sewage not nitrified by pure cultures of the organisms?

In April 1914, Beesley published some results of "Experiments on the Rate of Nitrification".* His conclusion was, after experimenting with numerous nitrogenous substances, typical of the products of albuminoid hydrolysis, that when exposed to nitrifying action under identical conditions, the rates of nitrification by the above bacteria are in all cases, equal, for

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*J. Chem. Soc. 105 and 106, 1015. 1914.

the specific reaction involved, i.e., from ammonia to nitrites, etc. That is, he found that the complexity of the molecule played but little part in affecting the rate of nitrification.

Sewage itself, however, contains only partially broken down proteids. The following table* shows the resultant substances when bacteria contained in sewage, act on the proteids.

Albumin
Fibrin give
Casein

Glycine, $CH_2(NH_2)COOH$

Leucin, $C_5H_{10}(NH_2)COOH$

$$\text{Tyrosin}\ \begin{matrix}CH_2C_6H_4OH\\CH(NH_2)COOH\end{matrix}$$

$$\text{Aspartic acid}\ \begin{matrix}CH_2COOH\\CH(NH_2)COOH\end{matrix}$$

$$\text{Asparagin}\ \begin{matrix}CH_2CO(NH_2)\\CH(NH_2)COOH\end{matrix}$$

$$\text{Glutaminic acid}\ CH_2\begin{matrix}CH(NH_2)COOH\\CH_2COOH\end{matrix}$$

etc.

and these break down further:

Glycin to ammonia and acetic acid.

Leucin to ammonia and isocaproic acid.

Tyrosin to phenol and aromatic acids.

Aspartic to ammonia and succinic acids.
Asparagin to ammonia and succinic acids.
Glutaminic to ammonia and succinic acids.

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*Sewage Disposal. Kinnicutt, Winslow and Pratt. 107. 1908.

All this is caused by bacterial action. Yet we know that nitrifying bacteria cannot attack the protein directly, in fact, if protein exists in too great concentration, the nitrifiers will not function. Here in part is the explanation of why pure cultures of the nitrifying organisms attacked the sterile sewage so slightly. The small amount of nitrites observed can probably be accounted for entirely by the urea and ammonia present, both of which are attacked and nitrified by these organisms.

Similar experiments with the pure culture of the nitrifiers and sterile sewage were made. Enough of the sterile sludge was added to bring the specific gravity of the mixture up to that existing in the tank sludge. (1.03). Results showed that the organic matter of the sterile sludge does not aid the nitrifying bacteria.

Mention has been made before, in this article and others*, of a certain phenomenon occurring in tanks of this nature. During the first part of the aeration period, a well clarified effluent, due to action on oxidixable matter, both colloid and crystalloid, results; then the real nitrification occurs. However, with the process running continuously these stages can not be differentiated. This would seem to show that either the flora and fauna of the tank, other than the nitrifying organisms are responsible for a change, which can only be regarded as a preliminary step in the

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*J. Soc. Chem. Ind. 33, 523-539-1122,1124. 1914.

nitrification process. It might be observed that the effluent obtained from the tank after a short aeration, while well clarified and stable, is not always completely nitrified.

Since the futility of trying to secure pure cultures of the non-bacterial population, for study, has been shown, studies with the view of eliminating the unnecessary organisms, must necessarily proceed from the bacterial side. If the same results are obtained with a mixed culture of bacteria isolated from the sludge as were obtained in the original process, the reasonable conclusion will be drawn that the non-bacterial population is not directly concerned in the preliminary changes or in the nitrification of the effluent.

In order to determine the function of the other flora its isolation was first undertaken.

From samples of sludge taken on three separate occasions, after the necessary dilution, fifteen agar plates were poured and incubated at 30° for forty-eight hours. From all these plates, the colonies appearing the most consistently were chosen and classified according to the chart of the Society of American Bacteriologists. Copies of these sheets are on file in the Water Survey. The group numbers of these forms are:

Nos.	
1	111.2223022
2	111.2222032
3	111.2222033
4	111.2222034

Nos.	
5	111.2222022
6	111.2222012
7	222.4444614
8	111.2223012
9	111.222-022
10	111.2222818
11	212.2224432
12	221.2223532
13	212.222-432

Of the total, four were non-spore formers, two were facultative anaerobes, almost all attacked sugars, forming no gas and very little acid. Nearly all hydrolysed starch, and attacked the casein in milk tubes. As can be readily seen, these forms belong, with four exceptions, to the B. subtilis group of aerobic spore formers which attack protein without creating a nuisance. The bacteria were shown to belong to a specific group of aerobic, spore-forming, protein-attacking bacteria, which predominate in the process, so long as aerobic conditions persist.

A representative "typical" flora having been demonstrated and obtained, the experiments on the nitrification of sterile sewage, by pure cultures of nitrifiers, in the presence of sterile organic matter, were repeated - only this time, in addition, a mixed inoculation of the above isolated forms, was added. The

whole was then aerated. Previous examination showed the ammonia content of the sterile sewage to be about 18 to 22 p.p.m.

At the end of six hours aeration, in flask 1, containing originally 20 p.p.m. of ammonia nitrogen and inoculated as given above, 18 p.p.m. of nitrate and only the barest trace of nitrite, with no ammonia, was found. Again the sterile check showed no nitrification.

Another flask, 2, exactly similar to the above, excepting that no nitrate organisms were inoculated, was also set up. Complete or rather almost complete change of the ammonia to nitrites was observed, with no nitrates.

In a separate flask, 3, an inoculation of the flora, minus the nitrifying organisms, was run. Examination showed traces of nitrites, with no nitrates - but that same clarification of the sewage effluent, noted above, was observed.

We feel that the above experiments have shown:

1. That the major portion of the nitrification proper, is due to two organisms, which work together in nitrifying the effluent.

2. That what might be called a "typical flora", is present in the sludge and is responsible for the first stage of the nitrification.

3. That in producing the stable and nitrified effluent, the non-bacterial population of the tank plays no direct part.

In contact and other bacterial beds, the function of

the larger forms is thot to be two-fold - to keep the bed "open" and to keep down extraneous "bacteria". In the process under consideration here, since there is no bed to keep "open", and since it has been shown that these forms are not necessary to secure nitrification, the only possible role left for them seems to be that of keeping down the extraneous forms. This is a mere matter of conjecture, however. Further work might be done along these lines.

On the basis of these experiments, we feel justified in concluding that in this process, the bacterial flora, other than nitrifiers, which predominates in the sludge, directly takes the place of the preliminary treatment employed in other methods.

CONCLUSIONS

The result of this investigation has shown that in the process of sewage purification, under consideration, the following is true:

1. A large, consistent reduction of the total number of bacteria in the sewage is obtained.

2. The actual stabilization process is due to a typical aerobic bacterial flora which gains almost complete ascendency. The other inhabitants are largely incidental.

3. The actual nitrification is accomplished by the two typical known nitrifiers - "Nitrosomonas" and "Nitrobactor".

In conclusion the author wishes to thank the following for their interest and assistance throughout this work:

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